PHYLOGENETIC ANALYSIS OF MORPHOLOGICAL CHARACTERS IN *OURISIA* (PLANTAGINACEAE): TAXONOMIC AND EVOLUTIONARY IMPLICATIONS[1]

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Abstract

Ourisia Comm. ex Juss. (Plantaginaceae; Scrophulariaceae s.l.) is a genus of herbaceous or suffruticose species that occur largely in high-elevation habitats of Andean South America, New Zealand, and Tasmania. Recent molecular phylogenetic data have clarified evolutionary relationships within *Ourisia* and provided high support for the recognition of two subgenera based primarily on habit, i.e., *Ourisia* subg. *Ourisia* (25 herbaceous species) and *Ourisia* subg. *Suffruticosae* Meudt (three suffruticose species). To investigate the utility of morphological data in comparison to molecular data for phylogeny and subgeneric taxonomy, phylogenetic hypotheses were generated using 20 morphological characters for all 33 taxa of *Ourisia* using *Melosperma* Benth. as an outgroup. Additional phylogenetic hypotheses were generated by combining the morphological data with molecular data for the 29 species (plus *Melosperma*) for which the latter were available. With respect to phylogeny, morphological data are congruent with molecular data but generally provide less resolution. Morphological data are informative regarding infrageneric taxonomy and show that at least six morphological characters in addition to habit support the delimitation of the two subgenera. Finally, morphological characters of interest were traced onto the molecular phylogeny. Evolution of these and other characters are discussed in light of the various phylogenetic results.

Key words: Character evolution, infrageneric taxonomy, morphology, *Ourisia*, phylogeny, Plantaginaceae, Scrophulariaceae s.l.

Resumen

Ourisia Comm. ex Juss. (Plantaginaceae; Scrophulariaceae s.l.) es un género de especies herbáceas o sufruticosas que crecen predominantemente en hábitats de grandes altitudes de los Andes de Sudamérica, Nueva Zelandia y Tasmania. Datos filogenéticos recientes han aclarado las relaciones evolucionarias dentro de *Ourisia* y han dado un gran respaldo al reconocimiento de dos subgéneros basados principalmente en hábito, es decir, *Ourisia* subg. *Ourisia* (25 especies herbáceas) y *Ourisia* subg. *Suffruticosae* Meudt (tres especies sufruticosas). A fin de investigar la utilidad de los datos morfológicos en comparación con los datos moleculares de filogenia y taxonomía subgenérica, se generaron hipótesis filogenéticas usando 20 caracteres morfológicos para los 33 taxa de *Ourisia*, con *Melosperma* Benth. como grupo externo. Se generaron otras hipótesis filogenéticas combinando los datos morfológicos con los datos moleculares de las 29 especies (más *Melosperma*) en las que se disponía de estos últimos. Respecto de la filogenia, los datos morfológicos son coherentes con los datos moleculares, pero por lo general ofrecen menos resolución. Los datos morfológicos son informativos respecto de la taxonomía infragenérica e indican que al menos seis caracteres morfológicos, además del hábito, respaldan la delimitación de los dos subgéneros. Por último, se trazaron a la filogenia molecular caracteres que resultan de interés. Se analiza la evolución de estos y otros caracteres a la luz de los diversos resultados filogenéticos.

Species of *Ourisia* Comm. ex Juss. (Plantaginaceae; Scrophulariaceae s.l.) are distributed throughout Andean South America, New Zealand, and Tasmania, largely in high-elevation habitats up to 5000 m but extending down to sea level especially at high latitudes. Within *Ourisia*, 28 species are currently recognized (Meudt, 2006) and have five-lobed calyces, tubular to tubular-funnelform corolla tubes, five

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corolla lobes, four didynamous stamens adnate to the corolla tube (a staminode may or may not be present), and a bicarpellate pistil. Many of these floral traits are common in other genera of Scrophulariaceae s.l. (Kampny, 1995; Kampny & Dengler, 1997; Judd et al., 2002), which includes the recently re-circumscribed family Plantaginaceae to which *Ourisia* belongs (APG II, 2003; Albach et al., 2005).

Species of *Ourisia* also have opposite leaves, a capitate to emarginate stigma, and loculicidal capsules with many tiny, reticulate seeds. Species may be suffruticose with numerous leafy branches arising from a woody base, or herbaceous with repent or decumbent, branching stems. Flowers may be solitary or arranged in pairs or whorls in racemose, bracteate inflorescences. Species of *Ourisia* have red to purple pigments that may be found to various degrees on leaves, bracts, peduncles, pedicels, and/or calyces. Although the suite of floral and vegetative characters mentioned above may be used to distinguish *Ourisia* from related genera, none of the characters by themselves are unique to, or diagnostic for, the genus.

Phylogenetic analyses based on chloroplast and nuclear DNA sequence data have recently been performed to elucidate the biogeography and relationships among species of *Ourisia* (Meudt & Simpson, 2006). One interesting result from these analyses was high support for two clades within the genus that can be distinguished morphologically simply by habit. This, in part, led to the description of two subgenera within *Ourisia*: *Ourisia* subg. *Ourisia*, comprising all 25 herbaceous species, and *Ourisia* subg. *Suffruticosae* Meudt, with the three suffruticose species (Meudt, 2006). This infrageneric taxonomy contrasts with previous treatments such as that of Poeppig and Endlicher (1835; Table 1) followed by most subsequent workers, in which *Ourisia* subg. *Ourisia* consisted of species with irregular calyces and *Ourisia* subg. *Dichroma* (Cav.) Poepp. & Endl. of species with regular calyces.

The main goals of the present study were to analyze an array of morphological characters to: (1) investigate their significance with respect to the phylogeny and subgeneric taxonomy of *Ourisia*, and (2) understand patterns of evolution within *Ourisia* for specific morphological characters. To this end, a phylogenetic approach was employed, in which novel morphological data and previously published molecular phylogenetic data from Meudt and Simpson (2006) were analyzed separately and together. Morphological characters of interest were also optimized onto one of the molecular phylogenetic trees in order to assess their evolution within *Ourisia*.

MATERIALS AND METHODS

TAXA INCLUDED

Ingroup. Morphological data were collected from all 33 taxa (28 species) in *Ourisia*. Previously published molecular data (Meudt & Simpson, 2006) were available for only 29 taxa (26 species) because high-quality DNA could not be extracted from four taxa (i.e., *O. breviflora* Benth. subsp. *uniflora* (Phil.) Meudt, *O. biflora* Wedd., *O. coccinea* (Cav.) Pers. subsp. *coccinea*, *O. fuegiana* Skottsb.).

Outgroup. Historically, *Ourisia* has been placed in tribe Digitalideae or Veroniceae (see Meudt, 2006 for an overview), but a recent molecular phylogenetic study of Plantaginaceae has shown that these tribes are not monophyletic and *Ourisia* is not closely related to any of the genera from these tribes (Albach et al., 2005). Instead, *Ourisia* was found to be part of a clade that contains at least 10 other genera from the tribes Gratioleae (*Bacopa* Aubl., *Gratiola* L., *Mecardonia* Ruiz & Pav., *Otacanthus* Lindl., *Scoparia* L., and *Stemodia* L.), Angelonieae (*Angelonia* Humb. & Bonpl. and *Basistemon* Turcz.), and Melospermeae (*Melosperma* Benth. and *Monttea* Gay) (Albach et al., 2005). In their combined analysis of four DNA markers (Albach et al., 2005), *Ourisia* was sister to Angelonieae–Melospermeae but with very little support (< 50 bootstrap percentage [BP]; 0.88 posterior probability [PP]); Gratioleae in turn was monophyletic and sister to *Ourisia* and Angelonieae–Melospermeae. Of the four genera in this latter group, *Melosperma* is morphologically most similar to *Ourisia* (especially the suffruticose species) in its basally woody and creeping (to subshrub) habit, sessile leaves, presence of hypogynous disc, and solitary, axillary flowers. The presence of these characters allows more comparable scoring of morphological characters than any other genus. In addition, unlike *Angelonia*, *Monttea*, and some species of *Basistemon*, the flowers of *Melosperma* and *Ourisia* do not produce oil as a reward for pollinators. Finally, *Melosperma* is a monotypic genus endemic to the southern Andes, where *Ourisia* is postulated to have arisen (Meudt & Simpson, 2006). For these reasons, *Melosperma* appears to be the best choice for the outgroup until all genera in the family (and especially in Gratioleae) are included in a phylogenetic analysis and a sister group to *Ourisia* is elucidated with high support. A thorough analysis and discussion of character evolution including more genera from the clade to which *Ourisia* belongs, and indeed from Plantaginaceae as a whole, are clearly warranted but are beyond the scope of this study.

Table 1 lists the geographical area of distribution, habit, authorities, representative voucher information,

Table 1. Taxa sampled for this study, including geographical area, habit, authorities, voucher information, and subgeneric classification.

Geographical area and habit	Species	Representative voucher	Subgeneric treatment: Poeppig & Endlicher, 1835	Subgeneric treatment: Meudt, 2006
South America				
North-central Andean herbaceous taxa				
	Ourisia biflora Wedd.	Bolivia, La Paz, *Mandon s.n.* (P); Peru, Puno, *Stafford 1112* (F)	*Ourisia*	*Ourisia*
	Ourisia chamaedrifolia Benth.	Peru, Cusco, *Meudt & López 052* (TEX); Venezuela, Mérida, *Dorr & Barnett 5228* (NY)	*Dichroma*	*Ourisia*
	Ourisia cotapatensis Meudt & S. Beck	Bolivia, La Paz, *Beck 22777* (LPB); Bolivia, La Paz, *Beck 25989* (LPB)	*Dichroma*	*Ourisia*
	Ourisia muscosa Benth.	Colombia, Caldas, *Rangel 1816* (NY); Peru, Ancash, *Refulio et al. 23* (TEX)	*Dichroma*	*Ourisia*
	Ourisia pulchella Wedd.	Bolivia, Cochabamba, *Brooke 6271* (BM); Bolivia, La Paz, *Kessler 3397* (TEX)	*Ourisia*	*Ourisia*
Southern Andean herbaceous taxa				
	Ourisia alpina Poepp. & Endl.	Argentina, Neuquén, *Rossow 1367* (NA); Chile, IX Región, *Meudt et al. 040* (TEX)	*Dichroma*	*Ourisia*
	Ourisia breviflora Benth. subsp. *breviflora*	Argentina, Tierra del Fuego, *Meudt & López 058* (TEX); Chile, XI Región, *Donat s.n.* (PH)	*Dichroma*	*Ourisia*
	Ourisia breviflora Benth. subsp. *uniflora* (Phil.) Meudt	Argentina, Río Negro, *Boelcke & Correa 5583* (SI); Chile, VIII Región, *Werderman 1348* (F)	*Dichroma*	*Ourisia*
	Ourisia coccinea (Cav.) Pers. subsp. *coccinea*	Argentina, Neuquén, *Rossow 2091* (NA); Chile, IX Región, *Montero 8508* (OS)	*Dichroma*	*Ourisia*
	Ourisia coccinea (Cav.) Pers. subsp. *elegans* (Phil.) Meudt	Chile, VIII Región, *Meudt & López 007* (TEX); Chile, X Región, *Hollermayer 322* (UC)	*Dichroma*	*Ourisia*
	Ourisia fragrans Phil.	Argentina, Río Negro, *Meudt & López 056* (TEX); Chile, X Región, *Sparre & Constance 10832* (UC)	*Dichroma*	*Ourisia*
	Ourisia fuegiana Skottsb.	Argentina, Tierra del Fuego, *Goodall 3437* (NA); Chile, IX Región, *Hicken-Reichert s.n.* (SI)	*Dichroma*	*Ourisia*
	Ourisia pygmaea Phil.	Argentina, Neuquén, *Correa 5711* (BAB); Chile, IX Región, *Meudt et al. 041* (TEX)	*Dichroma*	*Ourisia*
	Ourisia ruellioides (L. f.) Kuntze	Argentina, Tierra del Fuego, *Meudt & López 059* (TEX); Chile, VII Región, *Meudt & López 033* (TEX)	*Ourisia*	*Ourisia*
Southern Andean suffruticose taxa				
	Ourisia microphylla Poepp. & Endl.	Argentina, Neuquén, *Gentilli 212* (BAB); Chile, VII Región, *Meudt & López 036* (TEX)	*Dichroma*	*Suffruticosae*

Table 1. Continued.

			Subgeneric treatment	
Geographical area and habit	Species	Representative voucher	Poeppig & Endlicher, 1835	Meudt, 2006
	Ourisia polyantha Poepp. & Endl.	Chile, Región Metropolitana, *Gradjot 3464* (GH); Chile, VI Región, *Arroyo 201149* (TEX)	*Dichroma*	*Suffruticosae*
	Ourisia serpyllifolia Benth.	Chile, IV Región, *Morrison 17045* (UC); Chile, IX Región, *Meudt & López 035* (TEX)	*Dichroma*	*Suffruticosae*
Australia Tasmanian herbaceous taxa				
	Ourisia integrifolia R. Br.	Australia, Tasmania, *Collier 1117* (HO); Australia, Tasmania, *Meudt et al. 065* (TEX)	*Dichroma*	*Ourisia*
New Zealand New Zealand herbaceous taxa				
	Ourisia caespitosa Hook. f.	New Zealand, North Island, *Druce s.n.* (CHR); New Zealand, South Island, *Meudt & López 91* (TEX)	*Ourisia*	*Ourisia*
	Ourisia confertifolia Arroyo	New Zealand, South Island, *Dickinson et al. s.n.* (OTA); New Zealand, South Island, *Meudt & López 093* (TEX)	*Ourisia*	*Ourisia*
	Ourisia crosbyi Cockayne	New Zealand, South Island, *Meudt & López 089* (TEX); New Zealand, Stewart Island, *Petrie s.n.* (AK)	*Dichroma*	*Ourisia*
	Ourisia glandulosa Hook. f.	New Zealand, South Island, *Adams s.n.* (WELT); New Zealand, South Island, *Meudt & López 090* (TEX)	*Ourisia*	*Ourisia*
	Ourisia macrocarpa Hook. f. subsp. *calycina* (Colenso) Arroyo	New Zealand, South Island, *Meudt & López 085* (TEX); New Zealand, South Island, *Obrien 90/1* (AK)	*Dichroma*	*Ourisia*
	Ourisia macrocarpa Hook. f. subsp. *macrocarpa*	New Zealand, South Island, *Meudt & López 097* (TEX); New Zealand, South Island, *Petrie s.n.* (WELT)	*Ourisia*	*Ourisia*
	Ourisia macrophylla Hook. subsp. *lactea* (L. B. Moore) Meudt	New Zealand, South Island, *Kalin 675* (CANU); New Zealand, South Island, *Meudt & López 080* (TEX)	*Dichroma*	*Ourisia*
	Ourisia macrophylla Hook. subsp. *macrophylla*	New Zealand, North Island, *Meudt & López 067* (TEX); New Zealand, North Island, *Meudt & López 076* (TEX)	*Dichroma*	*Ourisia*
	Ourisia modesta Diels	New Zealand, South Island, *Druce s.n.* (CHR); New Zealand, Stewart Island, *Moore s.n.* (CHR)	*Ourisia*	*Ourisia*
	Ourisia remotifolia Arroyo	New Zealand, South Island, *Arroyo 73153* (CHR); New Zealand, South Island, *Meudt & López 094* (TEX)	*Ourisia*	*Ourisia*
	Ourisia sessilifolia Hook. f. subsp. *sessilifolia*	New Zealand, South Island, *Meudt & López 096* (TEX); New Zealand, Stewart Island, *Oliver 5* (WELT)	*Dichroma*	*Ourisia*

Table 1. Continued.

Geographical area and habit	Species	Representative voucher	Subgeneric treatment	
			Poeppig & Endlicher, 1835	Meudt, 2006
	Ourisia sessilifolia Hook. f. subsp. *splendida* (L. B. Moore) Arroyo	New Zealand, South Island, *Cockayne s.n.* (WELT); New Zealand, South Island, *Meudt & López 102* (TEX)	*Dichroma*	*Ourisia*
	Ourisia simpsonii (L. B. Moore) Arroyo	New Zealand, South Island, *Druce s.n.* (CHR); New Zealand, South Island, *Meudt & López 081* (TEX)	*Ourisia*	*Ourisia*
	Ourisia spathulata Arroyo	New Zealand, South Island, *Mark s.n.* (OTA); New Zealand, South Island, *Meudt & López 100* (TEX)	*Ourisia*	*Ourisia*
	Ourisia vulcanica L. B. Moore	New Zealand, North Island, *Buchanan 13513* (HO); New Zealand, North Island, *Meudt & López 071* (TEX)	*Ourisia*	*Ourisia*
Outgroup				
	Melosperma andicola Benth.	Chile, Región Metropolitana, *Arroyo & Humaña 206607* (TEX)	—	—

and subgeneric classification of all ingroup and outgroup taxa.

MOLECULAR DATA

The materials and methods for DNA extraction, amplification via polymerase chain reaction, cloning, sequencing, and alignment of sequences are described in detail in Meudt and Simpson (2006). The four markers sequenced were: the nuclear ribosomal DNA (nrDNA) internal transcribed spacer region (ITS; including ITS-1, 5.8S, and ITS-2) and part of the external transcribed spacer region (ETS), as well as two chloroplast DNA (cpDNA) regions (*matK* 3′ intron and *rps16* intron). All sequences from Meudt and Simpson (2006) were previously submitted to GenBank. Because tree topologies for the four individual molecular markers were largely congruent (Meudt & Simpson, 2006), they were combined into one matrix and treated as a single molecular data set for all analyses.

MORPHOLOGICAL DATA

Morphological data were scored from a total of 347 herbarium specimens, including two to 44 per species (median = 10, mean = 12.7), depending on geographical range, variability, and availability of specimens (Meudt, 2006). Due to the tendency of specimens to turn black upon drying, color data were only rarely obtained from the specimen itself and information supplied on the collection labels was frequently used. A newly relevant character for the genus, seed coat morphology, was added by scanning electron microscopy (SEM) of seeds (Meudt, 2006). Chromosome counts were taken from the literature (see Meudt, 2006: appendix 2). Morphological characters for *Melosperma andicola* Benth. were scored using descriptions from the literature (Rossow, 1985; Sérsic & Cocucci, 1999). Of the many characters that were studied, 20 were chosen for phylogenetic analysis based on general ease of scoring, ability to discern discrete, non-overlapping character states, and interest in tracing the evolution of that particular character. The resulting morphological data matrix of 20 characters for all 33 ingroup taxa plus *Melosperma* is given in Table 2; a detailed list of these characters and their character states is found in Appendix 1.

Twelve of the 20 morphological characters were variable in at least one taxon. Kornet and Turner (1999) reviewed and compared the seven different ways to code polymorphisms for phylogeny reconstruction. We followed their suggestion that, when the ancestral state is unknown, the preferred method is to

Table 2. Morphological characters included in this study and the character states for each species of *Ourisia* and the outgroup *Melosperma andicola*. Asterisks (*) indicate the four taxa that were not included in the molecular analyses and were thus deleted from the morphological matrix before combining the molecular and morphological data sets. Characters and character states are described in Appendix 1.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Melosperma andicola	0	0	–	0	0	–	0	2	01	0	1	0	0	0	–	01	–	0	–	–
Ourisia alpina	1	1	0	1	1	23	1	01	01	0	0	0	0	0	1	01	01	1	1	–
*Ourisia biflora**	1	1	0	01	1	1	0	0	01	1	0	0	1	0	0	1	0	–	–	–
Ourisia breviflora subsp. *breviflora*	1	1	0	01	1	12	1	01	01	0	0	0	0	1	2	1	01	1	1	–
Ourisia breviflora subsp. *uniflora**	1	1	0	01	1	1	1	0	1	0	0	0	0	0	12	01	01	1	1	–
Ourisia caespitosa	1	1	0	01	1	12	0	0	0	0	1	2	0	0	23	12	0	1	2	2
Ourisia chamaedrifolia	1	1	0	01	1	12	2	01	2	0	1	0	01	0	2	12	01	1	1	–
Ourisia coccinea subsp. *coccinea**	1	1	0	1	1	2	1	01	2	0	0	0	0	1	1	0	01	1	1	0
Ourisia coccinea subsp. *elegans*	1	1	0	1	1	23	1	01	2	0	0	0	0	1	1	0	01	1	1	–
Ourisia confertifolia	1	1	1	01	1	12	0	02	0	0	1	0	0	0	01	01	0	1	2	2
Ourisia cotapatensis	1	1	0	0	1	1	1	0	1	0	12	1	1	0	1	1	01	1	–	–
Ourisia crosbyi	1	1	0	2	1	23	1	0	0	0	1	2	0	0	01	01	0	1	2	2
Ourisia fragrans	1	1	0	01	1	2	2	0	01	1	0	0	0	0	0	2	0	1	1	–
*Ourisia fuegiana**	1	1	0	0	1	0	01	0	0	0	0	0	0	1	2	1	0	1	1	–
Ourisia glandulosa	1	1	1	01	1	12	0	0	0	0	12	0	0	0	01	01	0	1	2	2
Ourisia integrifolia	1	1	0	01	1	12	1	0	0	01	1	0	0	0	02	1	01	1	1	1
Ourisia macrocarpa subsp. *calycina*	1	1	0	1	1	3	1	0	0	0	1	2	01	0	01	1	0	1	2	2
Ourisia macrocarpa subsp. *macrocarpa*	1	1	0	1	1	3	0	0	0	0	1	2	0	0	01	01	0	1	2	2
Ourisia macrophylla subsp. *lactea*	1	1	0	1	1	3	1	02	0	0	1	2	1	0	1	01	0	1	2	2
Ourisia macrophylla subsp. *macrophylla*	1	1	0	1	1	23	1	0	0	0	1	2	1	0	1	01	0	1	2	2
Ourisia microphylla	0	0	–	0	0	–	1	2	01	1	0	0	0	0	0123	01	2	0	0	01
Ourisia modesta	1	1	0	0	1	1	0	01	0	0	–	1	1	1	0	01	0	1	3	2
Ourisia muscosa	1	1	0	0	1	0	02	01	0	1	1	3	1	0	02	01	01	1	4	–
Ourisia polyantha	0	0	–	0	0	–	1	2	2	1	1	0	0	0	3	01	2	0	0	–
Ourisia pulchella	1	1	0	01	1	1	0	01	01	0	0	0	1	0	1	–	–	–	–	–
Ourisia pygmaea	1	1	0	0	1	0	1	0	01	0	0	0	0	0	01	1	0	1	1	–
Ourisia remotifolia	1	1	0	1	1	12	0	02	0	0	2	2	0	0	0	01	0	1	2	2
Ourisia ruellioides	1	1	0	2	1	12	0	01	2	1	1	0	0	1	02	1	01	1	1	0
Ourisia serpyllifolia	0	0	–	0	0	–	1	2	1	1	1	0	01	0	1	01	2	0	0	–
Ourisia sessilifolia subsp. *sessilifolia*	1	1	1	1	1	23	01	01	0	0	2	2	0	0	0	0	0	1	2	2
Ourisia sessilifolia subsp. *splendida*	1	1	1	1	1	2	1	0	0	0	2	1	0	0	0	1	0	1	2	2
Ourisia simpsonii	1	1	1	1	1	12	0	0	0	0	12	0	0	0	0	1	0	1	2	2
Ourisia spathulata	1	1	1	01	1	12	0	01	0	0	1	0	0	0	01	1	0	1	2	2
Ourisia vulcanica	1	1	0	1	1	23	0	0	0	0	1	2	1	0	12	01	0	1	2	2

code polymorphisms as ambiguous. This was accomplished by allowing variable taxa to contain multiple states (e.g., a taxon that was variable for a presence/absence of a character was scored using the notation 01; see Appendix 1) and setting MSTAXA = polymorphic for analyses in PAUP* v4.0b10 (Swofford, 2002) and MacClade v4.05 (Maddison & Maddison, 2000).

To avoid introducing bias into the data set, we treated all morphological characters as unordered and used reductive coding for most characters (Wilkinson, 1995). However, we chose a "composite" approach for scoring six characters (four regarding vestiture, i.e., characters 8, 12, 15, and 17, plus calyx symmetry [character 7] and staminode [character 16]). For these six characters, there were three approaches to scoring: (1) considering each a single, multistate character with "absent" as one of the character states; (2) assuming two independent characters, A (presence/absence) and B (type of structure), and scoring taxa that are variable for the presence/absence character as 01 in character A and as missing in character B; and (3) assuming two independent characters, A (presence/absence) and B (type of structure), and scoring taxa that are variable for the presence/absence character as 01 in character A and as the present state (e.g., state 1 or state 2) in character B.

Method 1 was chosen for these characters because many taxa were variable for presence/absence of these structures, and this method seemed the most straightforward coding method that required no further assumptions regarding variable taxa. For the four vestiture characters in particular, this resulted in "glabrous" (i.e., trichomes absent) being considered a valid character state for vestiture, which we believe is a better choice than coding it as a separate, independent character. In general, trichomes in species of *Ourisia* may be found in varying densities on any given surface, from completely absent to forming a dense covering, suggesting that glabrous is a valid state at one end of the vestiture spectrum. In addition to the high occurrence of variability for presence/absence of trichomes in the data set, for two of the vestiture characters (8 and 17), taxa that had sessile glandular trichomes were always variable, 01. The staminode character 16 shows a similar pattern. This covariation (although not strict) suggests that the presence/absence and type "characters" may be biologically linked and should be treated as one character (Wilkinson, 1995). Method 1 also avoided creating extra characters with the majority of taxa scored as missing (i.e., ?), which can result in incorrect character optimization (Maddison, 1993; Strong & Lipscomb, 1999) or loss of information, or both. For example, two vestiture characters were constant when scored via method 2 because the majority of the taxa (30 and 31, respectively, out of 34 total) were scored as missing due to inapplicability or variability, making them phylogenetically uninformative and also very difficult to assess when mapped onto a molecular phylogeny.

For the reasons cited above and for consistency, we thus used the composite method 1 for scoring all four vestiture characters and for characters 7 and 16. Of course, as with reductive coding, composite coding has its deficiencies, especially when secondary losses occur (Strong & Lipscomb, 1999). In any case, choice of scoring method for these six characters does not appear to influence the phylogenetic analysis very much, as preliminary parsimony analyses using all three scoring options for these characters showed that there were only minor differences in tree topologies, tree scores, and bootstrap support for each of these methods (data not shown). Further empirical and simulation studies are needed, as the effect of these three coding options on the resulting phylogeny when taxa are variable for presence/absence does not appear to have been discussed in the literature regarding reductive versus composite coding (e.g., Maddison, 1993; Wilkinson, 1995; Strong & Lipscomb, 1999).

Two morphological matrices were assembled: one with all 33 ingroup taxa plus the outgroup, and a second with only the 29 ingroup taxa sampled for the molecular phylogeny, plus the outgroup. A combined data set was then assembled by adding this latter morphological matrix to the molecular data from Meudt and Simpson (2006) for 29 ingroup taxa (representing 26 species), plus *Melosperma*.

PHYLOGENETIC ANALYSES

The incongruence length difference (ILD) test (Farris et al., 1995) was used to test for significant heterogeneity of the molecular versus the morphological data sets by running 1000 permutations of the partition homogeneity test with MAXTREES set to 1000 for each comparison in PAUP*. The g1 statistic (Hillis & Huelsenbeck, 1992) was calculated in PAUP* as a measure of phylogenetic signal. Parsimony analyses were performed on all data sets in PAUP* with a simple heuristic search strategy, tree bisection-reconnection (TBR) branch swapping, 10 random addition replicates, MAXTREES set to 50,000, and gaps treated as missing data. One thousand bootstrap replicates were run with 10 random addition replicates and MAXTREES set to 1000. Bayesian analyses and one repetition of the maximum likelihood (ML) ratchet (Vos, 2003) were performed on the molecular data only using MrBayes v3.0b4 (Huelsenbeck & Ronquist, 2001) and PAUP*, respectively. The best model of evolution was chosen for the Bayesian and ML ratchet analyses using ModelTest v3.4 (Posada & Crandall, 1998). Because several most parsimonious trees (MPTs) resulted from the parsimony analysis of molecular data, and because these trees in turn were similar but not identical to trees from the Bayesian and likelihood ratchet analyses, one of these trees had to be chosen for optimization of morphological characters using MacClade. To this end, all resulting MPTs were scored via likelihood in PAUP* (using the best model of evolution chosen by ModelTest). Then the MPTs with the best ML score were compared to the Bayesian 50% majority rule tree and the likelihood ratchet best tree using topology and ML score as criteria for choosing one of the trees (see Results). For character optimization, the resolving option "show all most parsimonious states at each node" was used in MacClade.

RESULTS

PHYLOGENETIC ANALYSES OF MOLECULAR DATA

The molecular data set of 29 ingroup taxa plus *Melosperma* contained significant phylogenetic signal according to the g1 statistic (Table 3). Parsimony analysis resulted in 288 MPTs of length 1066 (Table 3; trees not shown). Replicate Bayesian analyses using the model HKY + G + I resulted in a 50% majority rule tree that was fully resolved except for four polytomies where branches had < 0.50 PP. One of the resolutions of this tree is shown in Figure 1, which has a similar but not identical topology to some of the MPTs from the parsimony

Table 3. Comparison of parsimony phylogenetic analysis statistics for the *Ourisia* molecular and morphological data sets analyzed for this study. CI, consistency index; RI, retention index; RC, rescaled consistency index.

Data set	Total no. of terminals	No. of aligned characters	g1 Test*	No. of MPTs	Tree length	No. of parsimony informative characters (%)	CI	RI	RC
Molecular only (4 DNA regions combined)	30	2689	−2.135491	288	1066	375 (13.9%)	0.820	0.835	0.685
Morphological only	34	20	−0.558530	19,840	170	20 (100%)	0.818	0.756	0.618
Morphological only[†]	30	20	−0.747964	> 50,000	158	20 (100%)	0.823	0.731	0.601
Combined (molecular plus morphological)	30	2709	−2.129626	9	1372	395 (14.6%)	0.832	0.819	0.681

* g1 Test critical value = − 0.09.

[†] Data set for the morphological matrix reduced to the 29 ingroup taxa plus *Melosperma*.

analysis. Trees from both analyses contained many of the same monophyletic groups of taxa, which in general were moderately to highly supported by parsimony BP values > 70% and PP values > 0.95 (BP and PP values are both shown on the Bayesian tree in Fig. 1). In these analyses, the three southern Andean suffruticose species *Ourisia microphylla* Poepp. & Endl., *O. polyantha* Poepp. & Endl., and *O. serpyllifolia* Benth. of *Ourisia* subg. *Suffruticosae* are highly supported as monophyletic (100 BP/1.0 PP) and sister to the herbaceous species of *Ourisia* subg. *Ourisia* (100 BP/1.0 PP), consistent with previous results (Meudt & Simpson, 2006).

Eighteen of the 288 MPTs composed two islands of trees that had the two best likelihood scores (−9644.32959 and −9644.65012, respectively). These MPTs were also the most similar to the Bayesian tree (Fig. 1; Bayesian tree scores range from −9676.07 to −9675.85) and the best tree from the ML ratchet (tree score −9640.93321, not shown). The main differences between these MPTs and trees from the Bayesian and ML ratchet are the relationships of some New Zealand species and the placement of Tasmanian *Ourisia integrifolia* R. Br. But, as was shown previously via parametric bootstrap, *O. integrifolia* cannot be rejected as the sister to the New Zealand clade (Meudt & Simpson, 2006). The Bayesian and likelihood ratchet trees therefore have topologies that appear to be representative of the evolutionary history of the genus. In addition, the best ML ratchet tree had a better ML score (−9640.93321) than all of the MPTs. Because the resolved Bayesian tree topology represents one of the possible resolutions of the polytomies in the best ML ratchet tree, this tree topology (Fig. 1) was chosen for use in character optimization.

PHYLOGENETIC ANALYSES OF MORPHOLOGY

The morphological data set contained phylogenetic signal according to the g1 statistic (Table 3). Parsimony analysis of the 20 morphological characters with all 33 ingroup taxa plus *Melosperma* resulted in 19,840 MPTs of length 170 (Table 3). The semi-strict consensus (or combinable component) tree of the MPTs (not shown; supplementary figure available from the author, as PDF online) shows all non-conflicting relationships and is a better representation of the data than the strict consensus tree due to the many MPTs with polytomies. In general, there is not much resolution in the semi-strict tree, but as with the analysis of molecular data (Fig. 1), there is (weak) support for *Ourisia* subg. *Suffruticosae* (61 BP). There is also high support for *Ourisia* subg. *Ourisia* (92 BP) and the sister relationship of *O. coccinea* subsp.

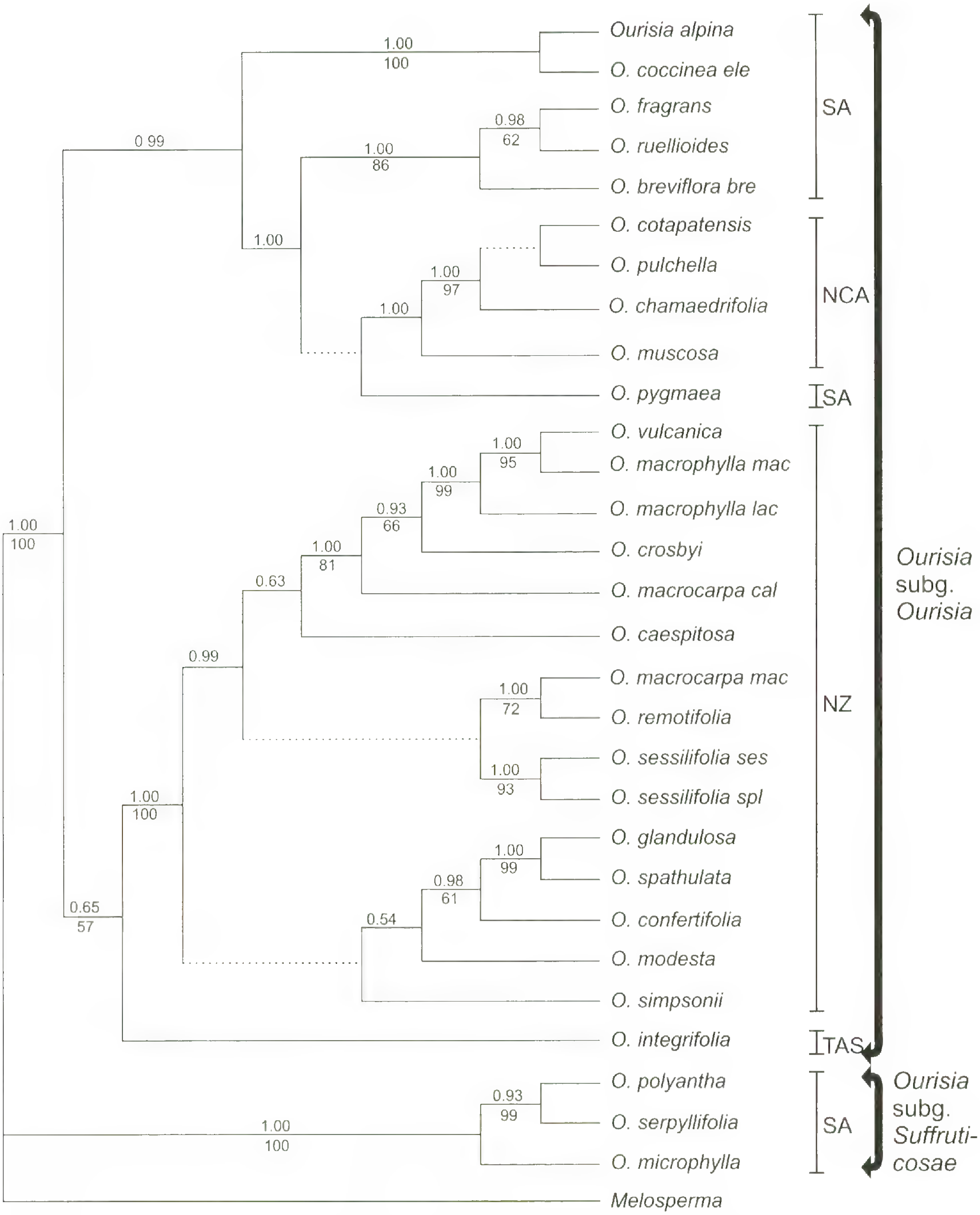

Figure 1. Bayesian phylogeny of the *Ourisia* molecular data set. One of the resolutions of the 50% majority rule consensus tree (which contained four branches with < 0.50 PP) of post-burn-in trees from one of the four replicates conducted for four million generations. This tree topology also represents one of the possible resolutions of the ML ratchet tree that had the highest ML score. Numbers above the branches indicate PP values, and numbers below the branches indicate BP values ≥ 50 from the parsimony analysis of the same data set. The four branches with < 0.50 PP (dashed lines) collapse in the 50% majority rule tree. Brackets and letters to the right of taxon names refer to geographic area (SA, southern Andes; NCA, north-central Andes; NZ, New Zealand; TAS, Tasmania); thickened lines show subgeneric classification. Subspecies abbreviations are as follows: O. coccinea ele, *O. coccinea* subsp. *elegans*; O. breviflora bre, *O. breviflora* subsp. *breviflora*; O. macrophylla mac, *O. macropylla* subsp. *macrophylla*; O. macrophylla lac, *O. macrophylla* subsp. *lactea*; O. macrocarpa cal, *O. macrocarpa* subsp. *calycina*; O. macrocarpa mac, *O. macrocarpa* subsp. *macrocarpa*; O. sessilifolia ses, *O. sessilifolia* subsp. *sessilifolia*; O. sessilifolia spl, *O. sessilifolia* subsp. *splendida*.

elegans (Phil.) Meudt and *O. coccinea* subsp. *coccinea* (82 BP). This latter taxon was one of four that was unable to be sequenced; the other three (*Ourisia biflora*, *O. breviflora* subsp. *uniflora*, and *O. fuegiana*) were unresolved in the herbaceous clade. Only two other branches representing relationships among some of the New Zealand species are resolved in this tree (not shown), but they receive < 50 BP. This general lack of support in morphologically generated phylogenetic hypotheses is not uncommon and is likely due to the small number of characters analyzed (Bremer et al., 1999). Reducing the morphological matrix to the 29 ingroup taxa plus *Melosperma* gave > 50,000 MPTs of length 158 (Table 3). The topologies of the semi-strict consensus tree of this analysis were similar to but slightly less resolved than that of the analysis with all 34 taxa included (not shown).

PHYLOGENETIC ANALYSES OF MOLECULAR AND MORPHOLOGICAL DATA COMBINED

The ILD test found significant heterogeneity for the pairwise comparison of the morphological data set with the molecular data ($P < 0.001$). Visual inspection of the topologies of the Bayesian tree (Fig. 1) and the semi-strict consensus tree of the parsimony analysis of morphological data (not shown) showed that they do not conflict with one another. Indeed, even tree topologies that are very similar can still show a significant result in the ILD test (Johnson & Soltis, 1998), and here this might be due to the large difference in number of characters between the two data sets (2689 vs. 20). As the effect of the addition of morphological data on the phylogeny of *Ourisia* was of interest, the molecular and morphological data sets were combined into one data matrix despite the heterogeneity found in the ILD test. This combined matrix, like the individual data sets that compose it, contained phylogenetic signal (Table 3) and resulted in nine MPTs of 1372 steps each (Table 3; the strict consensus tree is shown in Fig. 2 and is identical to the semi-strict tree). These trees were generally similar topologically to those of the molecular-only analysis and contained clades with similar BP values (compare Figs. 1 and 2). However, the combined trees did differ topologically with most analyses of molecular data alone (Bayesian, ML ratchet, and MPTs with the highest ML) by the placement of *O. muscosa* Benth. as the sister to all other herbaceous species of *Ourisia*. Of the clades that received > 50 BP in the combined analysis, seven had the same value (including 100 BP support for both subgenera), whereas eight increased by 1–8 BP and four decreased by 8–13 BP relative to support values for the same clades in the parsimony analysis of molecular data alone.

DISCUSSION

IMPLICATIONS OF ANALYSES OF MORPHOLOGICAL DATA ON SUBGENERIC TAXONOMY AND PHYLOGENY OF *OURISIA*

As expected, none of our analyses of the morphological data supported the delimitation of subgenera based on the characters used by Poeppig and Endlicher (1835), namely calyx symmetry (character 7), corolla curvature, and corolla lobe orientation (character 14). These latter two characters are not correlated with calyx symmetry in any species (except *Ourisia ruellioides*), and all three characters are homoplastic when mapped onto the phylogeny (data not shown). In contrast, delimitation of two subgenera within *Ourisia* based on habit (character 1; Meudt, 2006) is highly supported by phylogenetic analysis of morphological and combined data (e.g., Fig. 2). At least six other morphological characters, including leaf attachment (character 2), inflorescence type (character 5), calyx internal glandular vestiture (character 8), ovary vestiture (character 17), hypogynous disc (character 18), and seed type (character 19), support this infrageneric taxonomy. All of these characters (except seed type) have character states that are synapomorphies for the 25 species in *Ourisia* subg. *Ourisia*, i.e., herbaceous habit, petiolate leaves, bracteate inflorescences, glabrous or sessile glandular trichomes inside calyx and on the ovary, and a reduced hypogynous disc (characters 1, 2, 5, and 18 shown in Fig. 3).

Within *Ourisia* subg. *Ourisia*, the New Zealand herbaceous species are monophyletic in both the molecular and combined analyses (Figs. 1 and 2), and are linked by two synapomorphies: $2n = 48$ chromosomes (character 20) and New Zealand–type seed coat (character 19; with the exception of *O. modesta* Diels with autapomorphic *modesta*-type seeds). Support for most relationships within the New Zealand clade remain the same or are slightly higher when morphological and molecular data are analyzed together. Analysis of the combined data set shows that the Andean *O. alpina* Poepp. & Endl. and *O. coccinea* are sister to the Australasian species (Fig. 2), which contrasts with the Bayesian analysis of the molecular data that supports a monophyletic herbaceous Andean clade that is sister to the Australasian species (Fig. 1). There are no morphological synapomorphies for a herbaceous Andean clade. Finally, the addition of morphological data to the analysis does not support the monophyly of the four sampled north-central Andean species (Fig. 2). Three of these species—*O. cotapatensis* Meudt & S. Beck, *O. pulchella* Wedd., and *O. chamaedrifolia* Benth.—are strongly supported as monophyletic in both molecular (97 BP, 1.0 PP) and combined (88 BP)

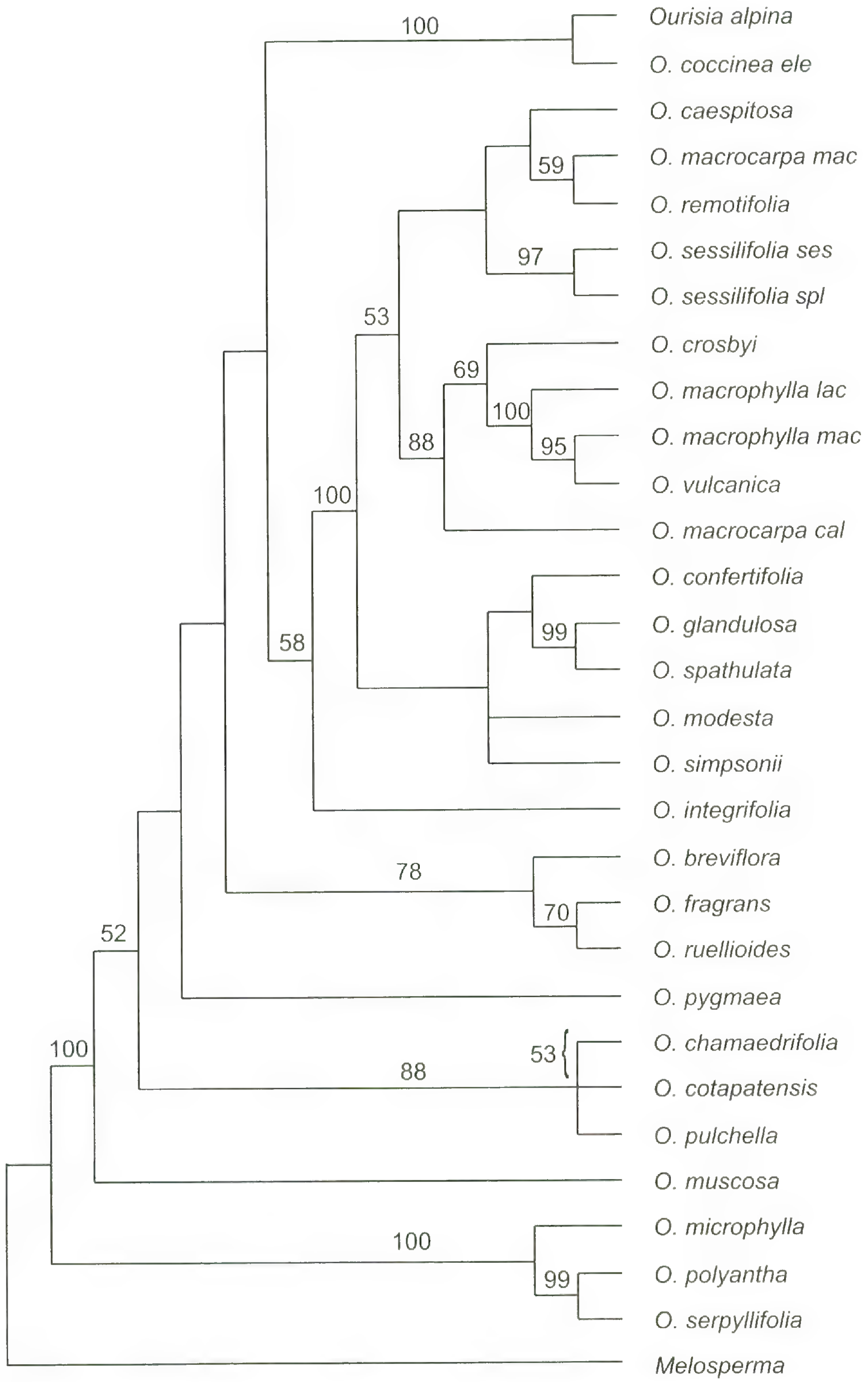

Figure 2. Strict consensus tree of the nine MPTs from parsimony analysis of the *Ourisia* combined (molecular plus morphological) data of 1372 steps. BP values > 50 are shown above the branches. The bracket { indicates 53 BP for the sister relationship of *O. chamaedrifolia* and *O. cotapatensis*, but these two species form part of a polytomy in the strict consensus tree shown here. Refer to Figure 1 for subspecies names.

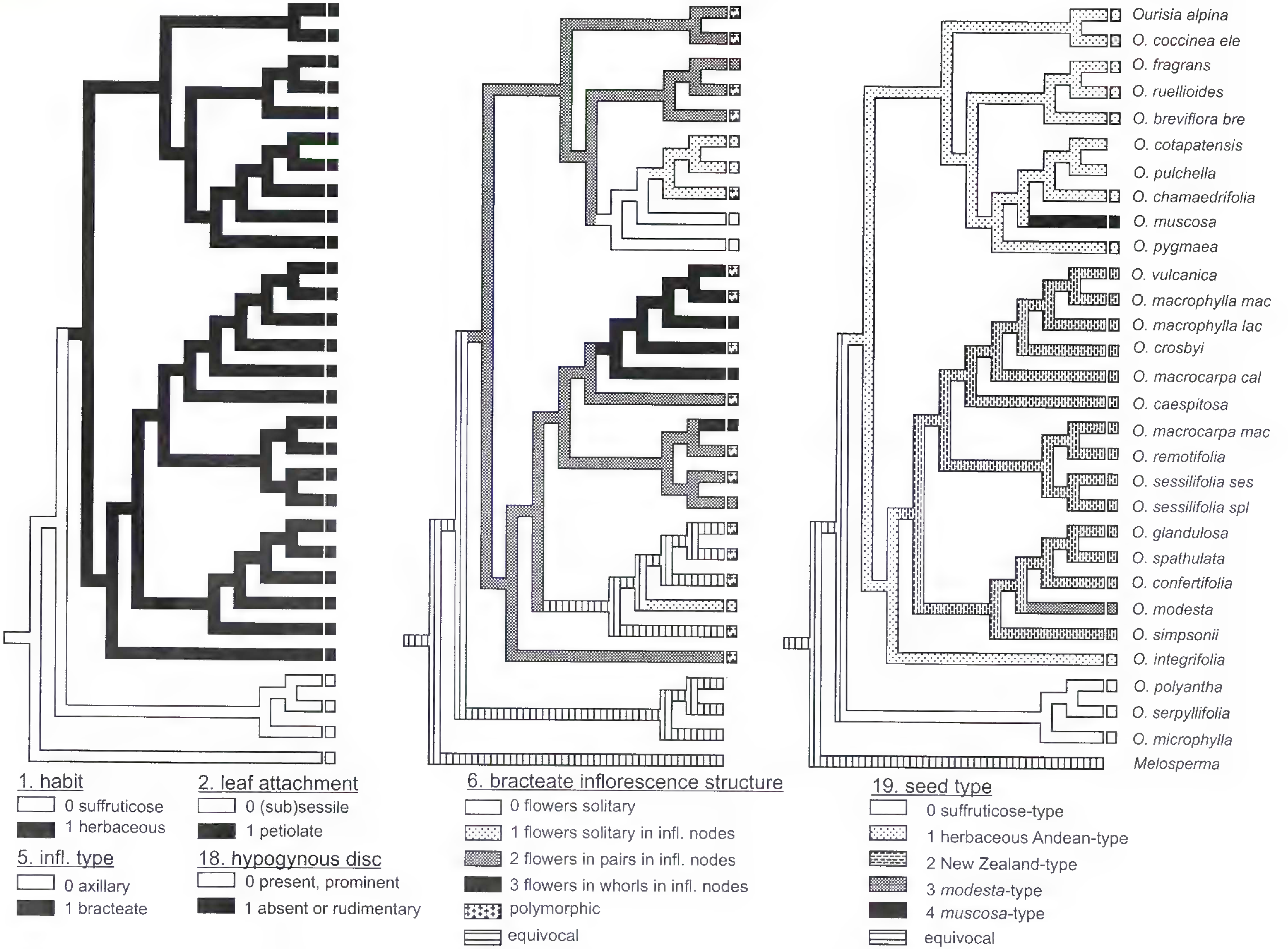

Figure 3. Morphological character evolution of selected characters in *Ourisia*. Characters 1, 2, 5, 18, and 19 support delimitation of two subgenera, *Ourisia* and *Suffruticosae*; character 6 is also shown. All characters are mapped onto the Bayesian tree of the molecular data set (Fig. 1). For a detailed explanation of characters and character states, see Appendix 1; for the character matrix, see Table 2. Refer to Figure 1 for subspecies names.

analyses. Whereas *O. muscosa* is strongly supported as sister to these three species in the molecular-only analysis (1.0 PP, Fig. 1), it does not form part of this clade in the combined analysis (Fig. 2). The north-central Andean species do not share any morphological synapomorphies, in part due to the unique morphology of *O. muscosa*, which is a minute species with solitary white, tubular, regular flowers and a dense vestiture of yellow trichomes inside the corolla tube that sets it apart from the other four species. *Ourisia muscosa* has two characters that are unique in the genus: calyces covered by a pair of subtending bracts and autapomorphic *muscosa*-type seeds. As *O. muscosa* reaches the highest elevation (5000 m) of any species of *Ourisia*, a plausible hypothesis for its unique characters is that there has been strong selection for morphological features adapted to these extreme environments.

Turning now to *Ourisia* subg. *Suffruticosae*, there are at least two possible synapomorphies for this clade (ovary with stalked glandular vestiture and suffruticose-type seeds, Fig. 3); however, the states for these characters in the outgroup are unknown. *Ourisia* subg. *Suffruticosae* shares at least five pleisiomorphic character states with the outgroup, *Melosperma*, namely woody habit, sessile leaves, solitary, axillary flowers, presence of a prominent hypogynous disc (characters 1, 2, 5, and 18 in Fig. 3), and stalked glandular hairs on the calyx (internally). These could represent the ancestral character states for *Ourisia*, but analyses with additional outgroups are needed.

Within *Ourisia* subg. *Suffruticosae*, analyses of morphological data show that relationships in this clade are either completely unresolved (morphology alone, not shown) or identical to those resolved in the molecular data tree (combined, Fig. 2). The three species have striking floral morphological differences but are very similar vegetatively. In fact *O. microphylla* and *O. polyantha* are only readily distinguished based on floral characters. However, *O. serpyllifolia* (not *O. polyantha*) was found to be sister to *O. microphylla* (Figs. 1, 2). Numerous autapomorphic characters distinguish *O. serpyllifolia* from all other species of *Ourisia*, including anthers with divaricate, explanate thecae, a style that is distinctively bent near the stigma, and stamens that are uniquely curved toward one another. Although all three suffruticose species occur in similar habitats in the southern Andes, *O. serpyllifolia* (29°–33°S, plus one disjunct locality at 36°S) is almost entirely allopatric to *O. polyantha* (33°–35°S) and *O. microphylla* (35°–38°S) (Meudt, 2006). It would be interesting to investigate whether differing pollinator faunas in these areas have resulted in specialization to different pollinators that are driving morphological divergence in these species, particularly in *O. serpyllifolia*.

PATTERNS OF EVOLUTION OF SELECTED MORPHOLOGICAL CHARACTERS OF INTEREST

Inflorescence type. Bracteate inflorescences in *Ourisia* subg. *Ourisia* have evolved from solitary, axillary flowers subtended by leaves found in *Ourisia* subg. *Suffruticosae* and the outgroup, *Melosperma* (character 5, Fig. 3). Within *Ourisia* subg. *Ourisia*, bracteate inflorescences with pairs of flowers in the inflorescence nodes appear to be ancestral (character 6, Fig. 3). Inflorescences with a reduced number of flowers and those with whorls of flowers have both evolved on separate occasions in the Andes and Australasia. In New Zealand, the seven taxa that can have inflorescences with whorled flowers in the inflorescence nodes belong to two different clades.

Seed morphology. *Ourisia* seeds are very small ($\leq 2.0 \times \leq 1.1$ mm) and have a glabrous, usually bireticulate seed coat that is superficially similar to seed coats in *Gratiola*, *Mecardonia*, *Melosperma*, *Bacopa*, and other genera of Gratioleae and other tribes (Meudt, 2006). Differences in seed coat morphology (character 19) can be used to distinguish subgenera in *Ourisia* (see Meudt, 2006 and Webb & Simpson, 2001 for SEM photographs of seeds). Suffruticose-type seeds are very distinctive from all other seed types and are only found in species of *Ourisia* subg. *Suffruticosae*. In contrast, there are four different seed types represented in species of *Ourisia* subg. *Ourisia*: herbaceous Andean–type, New Zealand–type, *muscosa*-type, and *modesta*-type. In our analysis, New Zealand–type, *modesta*-type, and *muscosa*-type seeds are all derived from the herbaceous Andean–type seeds (character 19, Fig. 3). A more detailed survey of seed coat morphological characters for related genera is needed to determine the ancestral seed type within *Ourisia*, which is equivocal.

Chromosome number. The base chromosome number (character 20) in *Ourisia* is $x = 8$, and all 12 New Zealand species have been determined to be hexaploids at $2n = 48$. The sole Tasmanian species, *O. integrifolia*, is $2n = 32$ (Hair & Arroyo, 1984), whereas the few Andean species that have counts (three out of 15) are $2n = 16$ (see Meudt, 2006), including *O. microphylla*, which also has been counted at $2n = 32$ (Rossow, 1993). When mapped onto the molecular phylogeny (not shown), chromosome number and ploidy levels show, as expected, an increasing trend from diploidy ($2n = 16$) to hexaploidy ($2n = 48$). This pattern of chromosome numbers may support the idea that a polyploidization event accompanied the colonization of Australasia by diploid South American ancestors of *Ourisia* (Meudt & Simpson, 2006). It is not known whether this event was due to allopolyploidy or autopolyploidy. If polyploidization occurred after long-distance dispersal of *Ourisia* to Australasia, perhaps autopolyploidy is more the likely scenario, as it only requires the dispersal of one individual. On the other hand, some species of *Ourisia* are interfertile and can produce natural (Meudt, 2006) or artificial (e.g., Sheader & Sheader, 2004) hybrids. More chromosome studies in *Ourisia* are needed to determine the extent to which polyploidy has occurred in the Andes and which Andean species have karyotypes most similar to the Australasian species.

Corolla characters. Several floral morphological characters are of interest with respect to character evolution within *Ourisia* (Fig. 4). Regarding internal corolla tube vestiture, trichomes have evolved four times from the ancestral glabrous state (character 12, Fig. 4). These transitions have occurred in both the north-central Andes and New Zealand. A monophyletic group of seven New Zealand species has three lines of trichomes inside the corolla tube, with the exception of *O. sessilifolia* Hook. f. subsp. *splendida* (L. B. Moore) Arroyo, which only has one line. The one line of hairs inside the corolla tube of *O. modesta* has apparently evolved independently within the clade that comprises the remaining New Zealand species, all with glabrous corolla tubes.

Red corollas have evolved on four separate occasions in Andean *Ourisia*, e.g., *O. polyantha*, *O. ruellioides*, *O. chamaedrifolia*, and *O. coccinea*, from white or light pink/light purple corollas (character 9, Fig. 4). These four species, which are not closely related to one another, have flowers that are also typically very long, with longer styles and stamens than those of other species of *Ourisia*. Long, red, tubular or tubular-funnelform corollas are often cited as indications of a hummingbird-pollinated syndrome (Grant & Grant, 1968). *Ourisia ruellioides* was studied with respect to its pollination biology and found to be self-compatible and strongly autogamous, with flowers that produce abundant nectar (Arroyo & Peñaloza, 1990). No pollinators were seen visiting this species during the course of their study (Arroyo & Peñaloza, 1990), which was conducted in the extreme south of Chile where pollinators may be less active and/or less abundant than at localities farther north (Arroyo et al., 1985).

The remaining species of *Ourisia* have light pink to light purple or white corollas with comparatively short corolla tubes. Many of these species have contrasting colors such as yellow or dark purple inside the corolla tube (character 11), often in the form of lines or spots, as well as some type of vestiture (characters 12 and 13) that may function as nectar guides for insect pollinators. Yellow inside the corolla tube is the most common state for this character and appears to be ancestral in *Ourisia* (character 11, Fig. 4). This contrasting color has been lost and regained in the Andes, but largely retained in most New Zealand species. Purple inside the corolla tube has also evolved once in the Andes (*O. cotapatensis*) and at least three times in New Zealand, i.e., *O. sessilifolia* and *O. remotifolia* Arroyo (purple only), *O. glandulosa* Hook. f. (purple and yellow), and *O. simpsonii* (L. B. Moore) Arroyo (purple and yellow) (character 11, Fig. 4). All species in New Zealand have small, white, weakly zygomorphic flowers that appear to be adapted to the unspecialized pollinator fauna there (Lloyd, 1985). For example, small generalist bees and flies have been observed pollinating the flowers of *O. macrocarpa* Hook. f. subsp. *calycina* (Colenso) Arroyo from New Zealand, which also contained trace amounts of nectar (Schlessman, 1986). Given the generalist alpine pollinator fauna of New Zealand, floral differences among the New Zealand species are particularly interesting and may suggest that pollinators have been a more important selective force than previously hypothesized in the evolutionary history of *Ourisia*.

Whether species of *Ourisia* with similar floral characteristics have similar pollinators, and whether these pollinators have driven the evolution of floral morphology in *Ourisia* are interesting questions that must be addressed by combining investigations of pollination biology and phenotypic selection with phylogenetics, as has been done recently in *Schizanthus* Ruiz & Pav. (Pérez et al., 2006) and *Penstemon* Schmidel (Wilson et al., 2006). It was shown in both of these studies that bird pollination has recently evolved numerous times (and at high elevation in *Schizanthus*) from bee-pollinated ancestors. These studies are particularly relevant here because *Penstemon* is a member of the same family as *Ourisia* and *Schizanthus* has a very similar distribution to *Ourisia* in the South American Andes. The finding that white, light pink, or light purple corollas appear to be ancestral and that red corollas appear to be derived in *Ourisia* (character 9, Fig. 4) also agrees with the hypothesis that, in general, insect-pollinated (and especially bee-pollinated), bell-shaped flowers are ancestral in Scrophulariaceae s.l. (Kampny, 1995). Although Scrophulariaceae s.l. is now known to be polyphyletic, this hypothesis can still be considered valid in the case of *Ourisia* because four of the tribes discussed by Kampny (1995) as having the ancestral bell-shaped flowers now form the core of a recircumscribed Plantaginaceae (Albach et al., 2005). There is also some evidence that bee pollination may even be ancestral in other closely related families in Lamiales (Kampny, 1995).

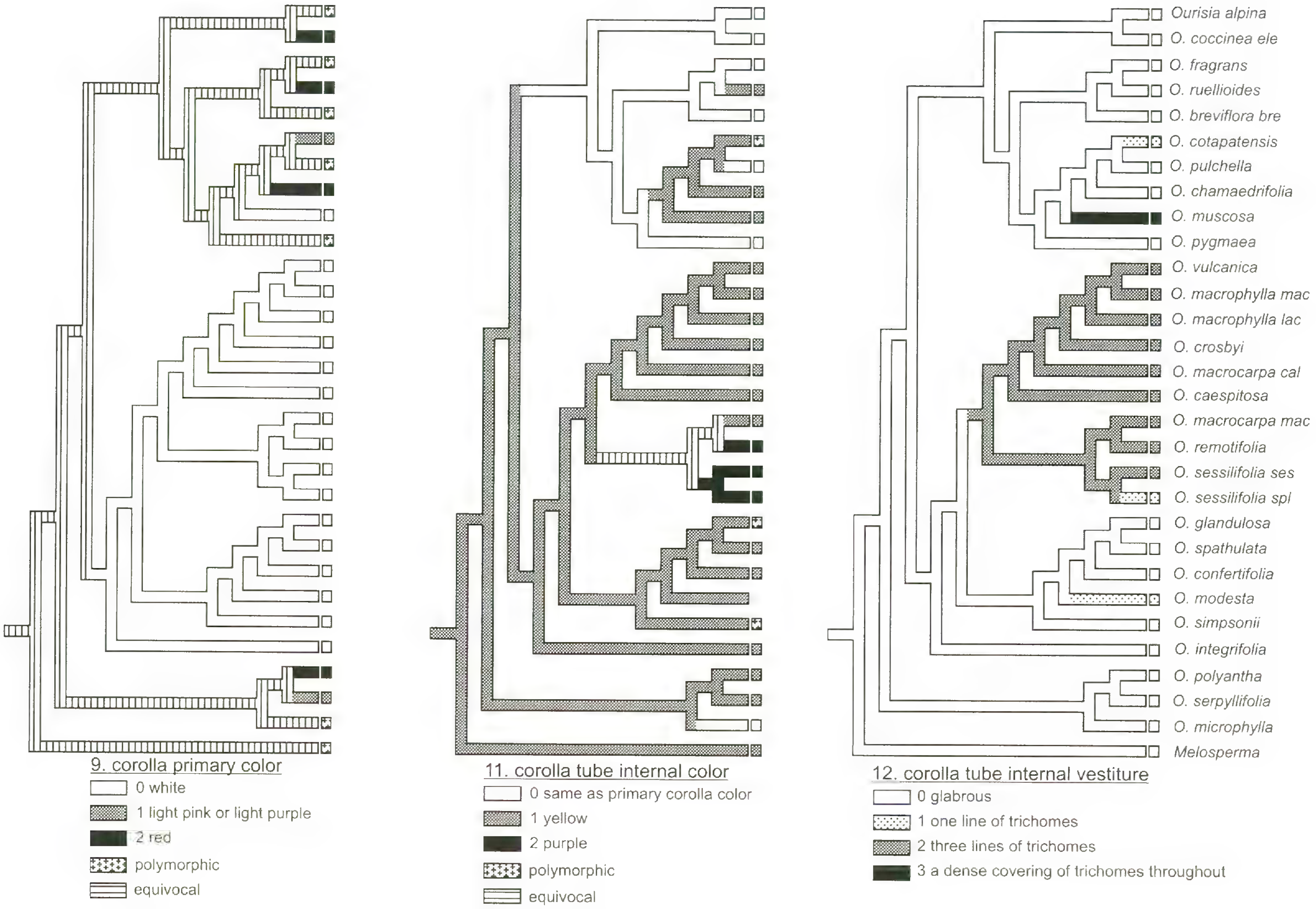

Figure 4. Morphological character evolution of selected characters in *Ourisia*. Characters 9, 11, and 12 represent selected floral characters discussed in the text. All characters are mapped onto the Bayesian topology of the molecular data set (Fig. 1). For an explanation of characters and character states, see Appendix 1; for the character matrix, see Table 2. Refer to Figure 1 for subspecies names.

In addition to being an ideal candidate for studying the evolution of floral features, breeding systems, and pollinators, *Ourisia* could be particularly informative regarding comparative studies between mountainous areas with differing pollinator faunas such as those in New Zealand and South America, or in the southern Andes alone, where at least three highly supported clades (Fig. 1) contain species with widely differing floral morphologies (e.g., *O. coccinea* and *O. alpina*; *O. breviflora*, *O. fragrans* Phil., and *O. ruellioides*; and *O. microphylla*, *O. polyantha*, and *O. serpyllifolia*).

SUMMARY

In summary, phylogenetic analyses of molecular and morphological characters both show high support for two monophyletic subgenera within *Ourisia*, with a notable number of morphological synapomorphies for the two subgenera. Morphological data supported or provided synapomorphies for some relationships within *Ourisia* subg. *Ourisia*, such as the New Zealand clade, but not for others, such as the herbaceous Andean species. Within the genus as a whole, and using *Melosperma* as an outgroup, analyses of morphological data elucidated several trends, including evolution from light-colored to red-colored corollas and glabrous to hairy corollas (both numerous times), simple to more complex inflorescences, and lower to higher ploidy levels. Seed coat morphology is particularly useful for defining groups within *Ourisia* and may be an important character for studies within Plantaginaceae as a whole, although future molecular and morphological phylogenetic studies for all plantaginaceous genera are necessary and warranted. *Ourisia* is an ideal candidate for further integrated studies regarding the evolution of floral features, breeding systems, and pollinators and could provide unique and comparative insights between species in mountainous areas of South America and Australasia.

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APPENDIX 1. Characters and states of the *Ourisia* morphological data set.

1. habit: 0, suffruticose with many branches arising from woody base; 1, herbaceous with repent stems.

2. leaf attachment: 0, sessile or subsessile; 1, petiolate.

3. petiole type: 0, petiole distinct from lamina; 1, petiole broad and difficult to distinguish where it begins and lamina ends. The common state among petiolate species of *Ourisia* is 0. This character was inapplicable for species with sessile leaves (character 2, state 0) and were thus coded as missing data in PAUP*.

4. leaf margin: 0, entire to subentire, or irregularly notched; 1, regularly crenate; 2, regularly serrate. A few taxa are variable, i.e., 01, with subentire to regularly crenate leaves.

5. inflorescence type: 0, solitary, axillary flowers subtended by leaves; 1, bracteate inflorescence.

6. inflorescence structure of bracteate inflorescences: 0, flowers solitary (never more than one flower per inflorescence); 1, flowers solitary in inflorescence nodes; 2, flowers in pairs in inflorescence nodes; 3, flowers in whorls in inflorescence nodes. There is variation within many taxa for inflorescence structure, resulting in taxa being coded polymorphic, either as 12 or 23. For example, *Ourisia alpina* usually has paired flowers in each node (state 2), but occasionally plants with whorled flowers in each node (state 3) can be found. This character was inapplicable for taxa that do not have bracteate inflorescences (character 5, state 0) and were thus coded as missing data in PAUP*.

7. calyx symmetry: 0, irregular, the three posterior lobes and two anterior calyx lobes divided to differing lengths; 1, regular or subregular, all calyx lobes divided all the way to base; 2, regular or subregular, all calyx lobes divided to ca. 1/2 the length of the calyx. Some taxa were variable for this character, i.e., 01 or 02. Regular calyces could have either all calyx lobes divided all the way to the base (i.e., a very short calyx tube, state 1) or all calyx lobes divided to ca. 1/2 the length of the calyx (i.e., a long calyx tube, state 2).

8. calyx internal glandular vestiture: 0, glabrous; 1, with sessile glandular trichomes; 2, with stalked glandular trichomes. Glandular trichomes may be sessile or stalked, but not both. All taxa with sessile glandular trichomes (state 1) were always variable, i.e., 01, and some taxa with stalked glandular trichomes were variable, i.e., 02.

9. corolla primary color: 0, white; 1, light pink or light purple; 2, red. Many Andean taxa are variable for white and light pink or purple corollas, i.e., 01, while other taxa have only white or only light pink/light purple corollas.

10. corolla symmetry: 0, zygomorphic; 1, regular or subregular. Corollas in the family are largely zygomorphic. The corolla symmetry of *Ourisia integrifolia* can be subregular to weakly zygomorphic and was thus coded as polymorphic, i.e., 01.

11. corolla tube internal color: 0, same as primary corolla color; 1, yellow; 2, purple. Some taxa with both yellow and purple inside the corolla tube were coded as polymorphic, i.e., 12.

12. corolla tube internal vestiture: 0, glabrous; 1, one line of trichomes; 2, three lines of trichomes; 3, a dense covering of trichomes throughout.

13. corolla tube vestiture at tube opening: 0, absent (glabrous); 1, present (trichomes more or less forming a ring). Some taxa are variable for this character, i.e., 01.

14. corolla lobe orientation: 0, widely spreading; 1, not spreading or only weakly spreading.

15. filament base vestiture: 0, glabrous; 1, patch of sessile glandular trichomes on two long stamens; 2, patch of sessile glandular trichomes on all four stamens; 3, sessile glandular trichomes in a ring around corolla tube base. Many taxa are variable for this character, with two states or, as in the case of *Ourisia microphylla*, even all four states present.

16. staminode: 0, absent; 1, present and rudimentary; 2, present as a fifth, fertile stamen. All taxa with a rudimentary staminode were always variable. The variable condition 01 was the most common. *Ourisia fragrans* always has five fertile stamens (state 2), while *O. chamaedrifolia* and *O. caespitosa* were coded as polymorphic for this character because, although they almost always have rudimentary staminodes, one to a few specimens were found with a fifth stamen, i.e., 12.

17. ovary vestiture: 0, glabrous; 1, with sessile glandular trichomes; 2, with stalked glandular trichomes. This vestiture character is similar to character 8, calyx internal glandular vestiture. Glandular trichomes may be sessile or stalked, but not both. All taxa with sessile glandular trichomes were always variable, i.e., 01.

18. hypogynous disc: 0, present, obvious, prominent; 1, absent or rudimentary, not prominent. The hypogynous disc is a ring of tissue around the base of the ovary that remains attached to the ovary when the corolla is shed. In some species, the disc is not prominent and can be difficult to assess in herbarium specimens. It is likely a nectary, but its anatomy needs to be investigated in more detail. Nectar has been found in the flowers of two herbaceous species of *Ourisia* that have been studied with respect to reproductive and pollination biology, including trace amounts in *O. macrocarpa* (Schlessman, 1986) and abundant nectar in *O. ruellioides* (Arroyo & Peñaloza, 1990). *Melosperma* was coded as 0 for this character because it contains a very similar hypogynous disc nectary (together with its sister genus *Monttea*: Sérsic & Cocucci, 1999).

19. seed type: 0, suffruticose-type; 1, herbaceous Andean type; 2, New Zealand–type; 3, *modesta*-type; 4, *muscosa*-type.

20. 2*n* chromosome number: 0, 16; 1, 32; 2, 48. Most of the South American species have chromosome numbers that are unknown for this character.